Response to Yoshizawa *et al.*

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The major source of surprise to us is the fact that none of the many groups who were/are working on the theme of nephritogenic antigens in acute poststreptococcal glomerulonephritis (APSGN) have published comparative studies. Our investigation was an attempt to remedy this situation.

Before addressing the points raised by Yoshizawa *et al.*, we wish to emphasize that there is now agreement on the data concerning streptococcal zymogen/proteinase (SPE B) in APSGN. Glomerular deposition of SPE B has been independently verified,[1] and has been confirmed by Yoshizawa *et al.* in their APSGN biopsies using antibody supplied by us (personal communication). In addition, a number of independent studies have shown antibody to SPE B to be an excellent marker of group A streptococcus infection in general (see Batsford *et al.*[2]) and it is probably the best available serological marker for APSGN.[3]

The second streptococcal component studied by us was glyceraldehyde-3-phosphate dehydrogenase (GAPDH), known to be identical to plasmin receptor from group A streptococcus[4] and sharing at least 98% homology to nephritis-associated plasmin receptor, as reported by Yoshizawa *et al.*[5] themselves. We found glomerular deposition of GAPDH in 3/17 biopsies and antibody to GAPDH in 5/47 serum samples from APSGN patients. These results demonstrate that more than one antigen may be involved in the pathogenesis of APSGN. The lower frequency of positive staining for GAPDH, found by us, is not easily explained by methodology; indirect immunofluorescence is actually more sensitive than direct staining and has been successfully used to detect glomerular deposition of many different antigens, as shown in myriad previous reports. The indirect technique is more likely to produce false-positive rather than false-negative results. Minor differences in mobility of fractions in sodium dodecyl sulfate-polyacrylamide gel electrophoresis reported between laboratories is a common phenomena of a technical nature, it does not constitute proof of real differences between antigens. Yoshizawa *et al.* used a quantitative immunoblot method to assess serum antibody; this is not state of the art, it should be confirmed by enzyme-linked immunosorbent assay testing. If Yoshizawa *et al.* are convinced of the existence of isoforms of GAPDH/plasmin receptor, they should perform complete sequencing of their antigen preparation, this task is not trivial but with modern technology it no longer poses great problems.

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Nephritogenic antigen for acute poststreptococcal glomerulonephritis

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To the Editor: We read with surprise the recent paper by Batsford *et al.*[1] on the nephritogenic antigen for acute poststreptococcal glomerulonephritis. The authors' data regarding glomerular deposition and serum antibody response to nephritis-associated plasmin receptor (NAPlr) or streptococcal glyceraldehyde-3-phosphate dehydrogenase in acute poststreptococcal glomerulonephritis patients were completely different from ours.[2,3] We detected serum anti-NAPlr antibody at high titer in 92% (46/50) of acute poststreptococcal glomerulonephritis patients, and positive glomerular staining was observed in 100% (25/25) of patients in the early stage by direct immunofluorescence staining with rabbit anti-NAPlr antibody.[3] We also showed that distribution of NAPlr deposition and plasmin activity were identical.[4] Discrepancy in histologic staining results is easily explained by methodology. Batsford *et al.*[1] used the indirect immunofluorescence staining with the different antibody that they made, whereas we used direct immunofluorescence staining for NAPlr because nonspecific staining increases and specific staining is difficult to assess by indirect methods. We suspect the discrepancy in serum reactivity against glyceraldehyde-3-phosphate dehydrogenase detected by ELISA was mainly because of the use of different antigens. The antigens differed in size (37–39 kDa (theirs) *vs* 43 kDa (ours)). We suspect the existence of two distinct isoforms of glyceraldehyde-3-phosphate dehydrogenase that would show differential migration by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and different immunologic reactivity.

Different isolation procedures may preferentially extract different isoforms, thereby causing the difference in results.

We agree that it is critical to compare the potential of streptococcal pyrogenic exotoxin B and NAPlr as nephritogenic antigens; however, for reliable comparison, the same antigen and the same antibodies should be used.

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VDR gene and urinary calcium excretion in nephrolithiasis

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To the Editor: We read the recent article by Loredo-Osti *et al.*[1] proposing that a major gene with relatively large effect on variation in urine calcium is segregating in French-Canadian families with stone disease, which could be localized using a quantitative trait locus mapping. We are studying the genetic basis of hypercalciuric nephrolithiasis in renal stone formers of North Indian origin. We have phenotyped stone formers into normocalciuric and hypercalciuric groups based on their 24 h urinary calcium ($U_{24}Ca$) excretion. The hypercalciuric patients and their families were investigated for linkage on chromosome 12, using five microsatellite markers (D12S87, D12S339, D12S361, D12S368, D12S90). A total of six families consisting of 18 affected and 19 unaffected individuals have been studied. Positive logarithm of odds score (1.69, 1.433, 2.1, and 1.88) were observed in four families and haplotype analysis narrowed the disease locus between markers D12S87 and D12S361. This region contains vitamin D receptor (*VDR*) gene locus, suggesting a possible linkage of *VDR* gene with hypercalciuric nephrolithiasis. We had earlier observed that nephrolithiatic and hypercalciuric nephrolithiatic patients, homozygous for *bb* genotype of *VDR Bsm*I restriction site, had significantly higher $U_{24}Ca$ (262.61 ± 24.28 mg/24 h and 387.34 ± 24.26 mg/24 h, $P<0.05$) than other genotypes. We also found that stone formers with homozygous (*FF*) genotype for *Fok*I restriction site showed significantly higher $U_{24}Ca$ excretion ($350.28 + 24.05$ mg/24 h, $P<0.05$).[2] Our results suggest that allelic variations in *VDR* gene may be associated with varied urinary calcium excretion in nephrolithiatic and hypercalciuric nephrolithiatic patients. However, further studies in different ethnic populations and with other variants of *VDR* gene are needed to confirm the role of *VDR* gene in renal stone disease.

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